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Wound healing and anti-inflammatory properties of *Ranunculus pedatus* and *Ranunculus constantinapolitanus*: A comparative study

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ABSTRACT

Ethnopharmacological relevance: In Turkish folk medicine *Ranunculus* species are used for wound healing and for the treatment of rheumatism. The present study was conducted to evaluate *in vivo* wound healing and anti-inflammatory properties of *Ranunculus pedatus* and *Ranunculus constantinapolitanus*.
Material and methods: In vivo wound healing activity of the extracts prepared from *Ranunculus pedatus* and *Ranunculus constantinapolitanus* was evaluated by linear incision and circular excision wound models. Hydroxyproline content of the treated tissues was also assessed. We also studied the anti-inflammatory activity using Whittle method with some modifications.
Results: Methanolic extract of *Ranunculus pedatus* showed significant wound healing effect both in incision (31.4%) and excision (55.74%) wound models. Methanolic extract of both *Ranunculus pedatus* and *Ranunculus constantinapolitanus* demonstrated anti-inflammatory activity with the inhibition value of 26.2% and 23.3% respectively, at the dose of 100 mg/kg. Hydroxyproline content of the tissues treated with the methanolic and aqueous extracts of *Ranunculus pedatus* and methanolic extract of *Ranunculus constantinapolitanus* were found to be significantly higher than that of the other extracts.
Conclusion: The experimental data revealed that *Ranunculus pedatus* showed significant wound healing and anti-inflammatory effect.

1. Introduction

Ranunculus L. (Ranunculaceae) is a widespread and temperate genus represented with 84 species in the Flora of Turkey (Davis, 1965, 1988; Güner, 2000). The genus *Ranunculus* is locally known as "basur otu, düğün çiçeği, katır nalı" in Turkey (Baytop, 1999).

Ranunculus species are used in traditional medicine as remedy for skin diseases (Gürhan and Ezer, 2004), antihemoroidal (Newall et al., 1996), wound healing (Zhu, 1990), antirheumatic (Sezik et al., 2001) and for the treatment of tuberculosis (Baytop, 1999), edema, abcesses and constipation (Gürhan and Ezer, 2004). According to literature survey, some plants belonging this genus have been shown to possess important biological properties such as antiviral (Li et al., 2005), antimicrobial (Barbour et al., 2004), anti-inflammatory (Cao and Meng, 1992; Prieto et al., 2003) and antiprotozoal (Orhan et al., 2006) activities. The methanolic extract of *Ranunculus constantinapolitanus* DC. was shown to possess anti-inflammatory activity using *in vitro* models of inflammation in a previous study (Fostok et al., 2009). However, there have been no reports on anti-inflammatory activity *Ranunculus pedatus* Waldst.&Kit. subsp. *pedatus*.

In previous phytochemical studies on *Ranunculus* species, flavonoids (Marston et al., 2006; Liang et al., 2008), saponins (Wegner and Hamburger, 2000), alkaloids (Zhang et al., 2007), fatty acids and organic acids (Chi et al., 2007) were isolated.

The aim of the present study is to evaluate the wound healing and anti-inflammatory activities of *Ranunculus constantinapolitanus* and *Ranunculus pedatus* by using *in vivo* experimental models.

2. Materials and methods

2.1. Plant material

Ranunculus pedatus Waldst.&Kit. subsp. *pedatus* and *Ranunculus constantinapolitanus* DC. were collected in May 2005 from Manisa-Spil and Yamanlar-İzmir, respectively. Both plants were identified by Ö. Seçmen from Ege University. The voucher specimens (herbarium numbers: 1349 for *Ranunculus constantinapolitanus* and 1364

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for *Ranunculus pedatus*) are deposited in the herbarium of the Faculty of Pharmacy, Ege University, İzmir, Turkey.

2.2. Preparation of plant extracts

Diethylether, *n*-hexane, ethyl acetate, methanol and water extracts were separately prepared from 20 g batches of the air-dried and powdered plant materials by extracting with 200 ml solvent at room temperature at 24 h. Then the solvents were evaporated to dryness *in vacuo* (60 °C). The yields of diethylether, *n*-hexane, ethyl acetate, methanol and water extracts of *Ranunculus pedatus* subsp. *pedatus* were 1.12%, 0.95%, 2.99%, 9.57%, and 12.01% and of *Ranunculus constantinapolitanus* were 0.95%, 1.05%, 3.52%, 10.21%, and 14.25%, respectively. All the extracts were stored at −20 °C.

2.3. Phytochemical screening

Phytochemical screening of crude extracts of the two plants was carried out using standard phytochemical methods (Harborne, 1998; Aguinaldo et al., 2005) (Table 1).

2.4. Biological activity tests

2.4.1. Animals

Male, Sprague-Dawley rats (160–180 g) and Swiss albino mice (20–25 g) were purchased from the animal breeding laboratory of Saki Yenilli (Ankara, Turkey).

The animals were left for 3 days at room conditions for acclimatization. They were maintained on standard pellet diet and water *ad libitum* throughout the experiment. A minimum of six animals were used in each group. Throughout the experiments, animals were processed according to the suggested European ethical guidelines for the care of laboratory animals.

2.4.2. Preparation of test samples for bioassay

Incision and excision wound models were used to evaluate the wound healing activity. For the *in vivo* wound models, test samples were prepared in an ointment base (vehicle) consisting of glycol stearate, 1,2 propylene glycol, liquid paraffin (3:6:1) in 1% concentration. 0.5 g of each test ointment was applied topically on the wounded site immediately after wound was created by a surgical blade.

The animals of the vehicle group were treated with the ointment base only, whereas the animals of the reference drug group were treated with 0.5 g of Madecassol® (Bayer, 00001199). Madecassol contains 1% extract of *Centella asiatica*.

For the assessment of anti-inflammatory activity, test samples were given orally to the test animals after suspending in a mixture of distilled H_2O and 0.5% sodium carboxymethyl cellulose (CMC). The control group animals received the same experimental handling as those of the test groups except the drug treatment was replaced with appropriate volumes of dosing vehicle. Indomethacin (10 mg/kg) in 0.5% CMC was used as reference drug.

2.4.3. Wound healing activity

2.4.3.1. Linear incision wound model. Animals, seven rats in each group, were anesthetized with 0.15 cc Ketalar®. The hairs on the dorsal part of the rats were shaved and cleaned with 70% alcohol. Two 5-cm length linear-paravertebral incisions were made with a sterile blade through the shaved skin at the distance of 1.5 cm from the dorsal midline on each side. Three surgical sutures were placed each 1 cm apart.

The ointments prepared with test samples, the reference drug (Madecassol®) or ointment base [glycol stearate:propylene glycol:liquid parafin (3:6:1)] were topically applied on the dorsal wounds in each group of animals once daily throughout 9 days. All the sutures were removed on the last day and tensile strength of previously wounded and treated skin was measured by using a tensiometer (Zwick/Roell Z0.5, Germany) (Suguna et al., 2002; Lodhi et al., 2006).

2.4.3.2. Circular excision wound model. This model was used to monitor wound contraction and wound closure time. Each group of animals (seven animals in each) was anesthetized by 0.01 cc Ketalar®. The back hairs of the mice were depilated by shaving. The circular wound was created on the dorsal interscapular region of each animal by excising the skin with a 5 mm biopsy punch; wounds were left open. Test samples, the reference drug (Madecassol®, Bayer) and the vehicle ointments were applied topically once a day till the wound was completely healed. The progressive changes in wound area were monitored by a camera (Fuji, S20 Pro, Japan) every other day. Later on, wound area was evaluated by using AutoCAD program. Wound contraction was calculated as percentage of the reduction in wounded area. A specimen sample of tissue was isolated from the healed skin of each group of mice for the histopathological examination (Sadaf et al., 2006).

2.4.3.3. Hydroxyproline estimation. Tissues were dried in hot air oven at 60–70 °C till consistent weight was achieved. Afterwards, samples were hydrolyzed with 6 N HCl for 4 h at 130 °C. The hydrolyzed samples were adjusted to pH 7 and subjected to chloramin T oxidation. The colored adduct formed with Ehrlich reagent at 60 °C was read at 557 nm (Woessner, 1961). Standard hydroxyproline was also run and values reported as μg/mg dry weight of tissue (Rasik et al., 1999).

2.4.4. Histopathology

The skin specimens from each group were collected at the end of the experiment. Samples were fixed in 10% buffered formalin, processed and blocked with paraffin and then sectioned into 5 μm sections and stained with hematoxylin and eosin (HE) and Van Gieson (VG) stains. The tissues were examined by light microscope

Table 1
Results of preliminary phytochemical screening.

Plant	Extract	Steroids	Triterpene	Alkaloids	Saponin	Tannin	Anthraquinone	Flavonoids	Sugars	Reducing Sugar	Coumarins	Starch
RP	Diethylether	–	–	+	–	–	–	–	–	–	–	–
	n-Hexane	–	–	–	–	–	–	–	–	–	–	–
	Ethyl acetate	–	–	–	–	–	–	+	–	–	–	–
	Methanol	+	+	–	–	–	–	+	–	–	–	–
	Water	–	–	–	+	–	–	–	–	–	–	–
RC	Diethylether	–	–	+	–	–	–	–	–	–	–	–
	n-Hexane	–	–	–	–	–	–	–	–	–	–	–
	Ethyl acetate	–	–	–	–	–	–	+	–	–	–	–
	Methanol	+	+	–	–	–	–	+	–	–	–	–
	Water	–	–	–	+	–	–	–	–	–	–	–

RP: *Ranunculus pedatus*; RC: *Ranunculus constantinapolitanus*; +: present; –: absent.

(Olympus CX41 attached Kameram® Digital Image Analyze System) and graded as mild (+), moderate (++) and severe (+++) for epidermal or dermal re-modeling. Re-epithelization or ulcus in epidermis; fibroblast proliferation, mononuclear and/or polymorphonuclear cells, neo-vascularization and collagen depositions in dermis were analyzed to score the epidermal or dermal re-modeling. Van Gieson stained sections were analyzed for collagen deposition. At the end of the examination, all the wound healing processes were combined and staged for wound healing phases as inflammation, proliferation, and re-modeling in all groups.

2.4.5. Anti-inflammatory activity

2.4.5.1. Acetic acid-induced increase in capillary permeability. Effect of the test samples on the increased vascular permeability induced by acetic acid in mice was determined according to Whittle method (Whittle, 1964) with some modifications (Yesilada and Küpeli, 2007). Each test sample was administered orally to a group of 10 mice in 0.2 ml/20 g body weight. Thirty minutes after the administration, tail of each animal was injected with 0.1 ml of 4% Evans blue in saline solution (i.v.) and waited for 10 min. Then, 0.4 ml of 0.5% (v/v) AcOH was injected i.p. After 20 min incubation, the mice were killed by dislocation of the neck, and the viscera were exposed and irrigated with distilled water, which was then poured into 10 ml volumetric flasks through glass wool. Each flask was made up to 10 ml with distilled water, 0.1 ml of 0.1 N NaOH solution was added to the flask, and the absorption of the final solution was measured at 590 nm (Beckmann Dual Spectrometer; Beckman, Fullerton, CA, USA). A mixture of distilled water and 0.5% CMC was given orally to control animals, and they were treated in the same manner as described above.

2.4.6. Statistical analysis of the data

The data on percentage anti-inflammatory and wound healing were statistically analyzed using one-way analysis of variance (ANOVA). The values of $p \leq 0.05$ were considered statistically significant.

Histopathologic data were considered to be nonparametric; therefore, no statistical tests were performed.

Table 2
Effect of the extracts of *Ranunculus pedatus* and *Ranunculus constantinapolitanus* on linear incision wound model.

Material	Extract type	Statistical mean ± S.E.M.	Tensile strength (%)
Vehicle		13.41 ± 2.81	1.1
Negative control		13.26 ± 2.92	–
Ranunculus pedatus	*n*-Hexane	14.80 ± 2.56	10.4
	Diethylether	13.75 ± 2.90	2.5
	Ethyl acetate	15.19 ± 2.14	13.3
	Methanol	17.62 ± 1.24	**31.4****
	Aqueous	15.89 ± 2.70	18.5
Ranunculus constantinapolitanus	*n*-Hexane	12.17 ± 2.41	–
	Diethylether	12.03 ± 2.32	–
	Ethyl acetate	13.76 ± 2.16	2.6
	Methanol	16.02 ± 1.65	19.5
	Aqueous	15.30 ± 2.08	14.1
Madecassol®		19.91 ± 1.06	**48.5*****

S.E.M.: standard error of the mean.
Percentage of tensile strength values: vehicle group was compared to negative control group; the extracts and the reference material were compared to vehicle group.
** $p < 0.01$.
*** $p < 0.001$.

Table 3
Effect of the extracts of *Ranunculus pedatus* and *Ranunculus constantinapolitanus* on circular excision wound model.

Material	Extract type	Wound area ± S.E.M. (contraction %)						
		0	2	4	6	8	10	12
Vehicle		19.49 ± 2.21	17.26 ± 2.43 (0.58)	15.84 ± 2.13 (4.06)	12.10 ± 1.75 (14.06)	9.10 ± 1.41 (13.58)	6.95 ± 1.23 (8.43)	3.05 ± 0.52 (3.48)
Negative control		19.62 ± 2.14	17.36 ± 2.15	16.51 ± 2.05	14.08 ± 1.63	10.53 ± 1.19	7.59 ± 1.84	3.16 ± 1.36
Ranunculus pedatus	*n*-Hexane	19.36 ± 2.19	16.24 ± 2.06 (5.91)	14.58 ± 2.22 (7.95)	10.62 ± 1.54 (12.23)	8.71 ± 1.52 (4.29)	6.20 ± 1.47 (10.79)	2.95 ± 0.49 (3.28)
	Diethylether	19.23 ± 2.12	15.78 ± 2.23 (8.57)	14.27 ± 1.72 (9.91)	10.40 ± 1.55 (14.05)	9.05 ± 1.62 (0.55)	6.13 ± 0.89 (11.79)	2.85 ± 0.41 (6.56)
	Ethyl acetate	19.87 ± 2.11	17.52 ± 2.05 (–)	15.10 ± 2.42 (4.67)	10.78 ± 1.94 (10.91)	9.86 ± 2.14 (–)	6.45 ± 1.52 (7.19)	3.62 ± 1.30 (–)
	Aqueous	19.54 ± 2.42	15.61 ± 2.54 (9.56)	13.26 ± 1.94 (16.29)	10.21 ± 1.57 (15.62)	9.15 ± 1.60 (–)	6.25 ± 1.06 (10.07)	2.21 ± 0.69 (27.54)
	Methanol	19.57 ± 2.17	15.29 ± 2.45 (11.41)	13.56 ± 1.87 (14.39)	9.65 ± 1.82 (20.25)	7.43 ± 1.86 (18.35)	4.79 ± 0.82 (**31.08**)*	1.35 ± 0.38 (**55.74**)**
Ranunculus constantinapolitanus	*n*-Hexane	20.13 ± 2.56	18.50 ± 1.82 (–)	15.81 ± 1.99 (0.19)	12.42 ± 1.71 (–)	8.89 ± 1.67 (2.31)	6.02 ± 0.59 (13.38)	2.17 ± 0.56 (28.85)
	Diethylether	19.41 ± 2.45	15.21 ± 1.44 (11.88)	13.25 ± 1.84 (16.35)	9.39 ± 1.42 (22.39)	7.52 ± 1.46 (17.36)	5.61 ± 0.56 (19.28)	2.51 ± 0.25 (17.70)
	Ethyl acetate	20.01 ± 2.36	15.84 ± 1.26 (8.23)	13.56 ± 2.03 (14.39)	9.17 ± 1.80 (24.21)	7.61 ± 1.19 (16.37)	5.49 ± 1.14 (21.01)	2.48 ± 0.15 (18.69)
	Aqueous	19.56 ± 2.02	16.45 ± 2.60 (4.69)	13.12 ± 1.78 (17.17)	9.92 ± 1.41 (18.02)	7.86 ± 1.08 (13.63)	6.03 ± 1.65 (13.24)	2.42 ± 0.34 (20.66)
	Methanol	19.55 ± 2.08	16.45 ± 2.02 (4.69)	14.25 ± 2.16 (10.04)	10.19 ± 1.81 (15.79)	7.81 ± 1.63 (14.18)	5.04 ± 1.16 (27.48)	2.10 ± 0.65 (**31.15**)*
Madecassol®		19.66 ± 2.15	15.62 ± 1.82 (9.50)	11.20 ± 1.53 (29.29)	6.52 ± 1.21 (**46.12**)**	3.40 ± 0.75 (**62.64**)**	0.79 ± 0.26 (**88.63**)***	0.00 ± 0.00 (**100.00**)***

S.E.M.: standard error of the mean.
Percentage of contraction values: vehicle group was compared to negative control group; the extracts and the reference material were compared to vehicle group.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

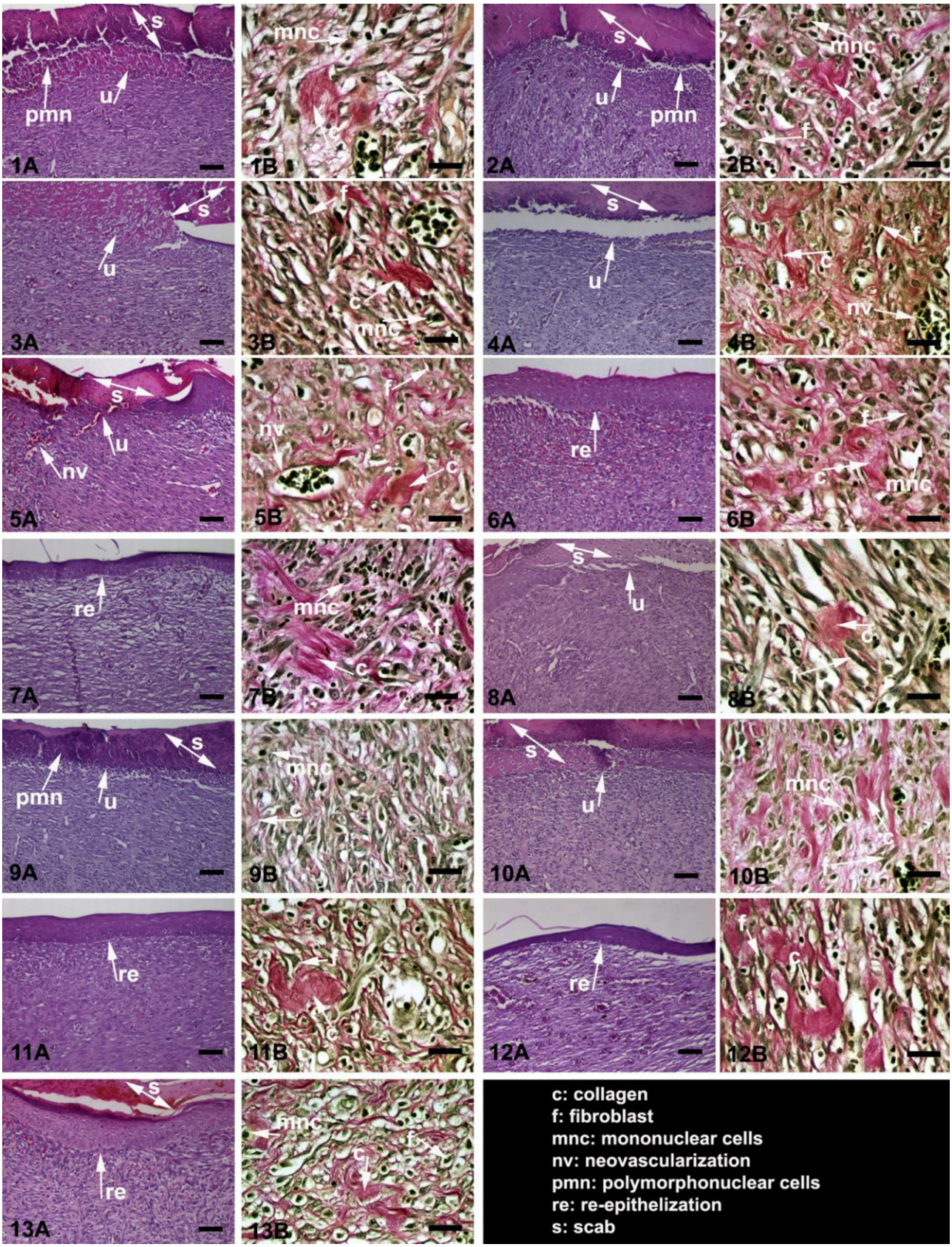

Fig. 1. Histopathological view of wound healing and epidermal/dermal re-modeling in the vehicle, negative control, extracts and reference ointment Madecassol® administered animals. Skin sections show the hematoxylin and eosin (HE) stained epidermis and dermis in A, and the dermis stained with Van Gieson (VG) in B. The original magnification was 100× and the scale bars represent 120 μm for figures in A, and the original magnification was 400× and the scale bars represent 40 μm for B. Data are representative of 6 animal per group. (1) Vehicle group, 10 day old wound tissue treated with vehicle, (2) negative control group, 10 day old wound tissue, untreated group, (3) *Ranunculus pedatus* diethylether group, 10 day old wound tissue treated with the diethylether extract of *Ranunculus pedatus*, (4) *Ranunculus pedatus n*-hexane group, 10 day old wound tissue treated with the *n*-hexane extract of *Ranunculus pedatus*, (5) *Ranunculus pedatus* ethyl acetate group, 10 day old wound tissue treated with the

Table 4
Wound healing processes and healing phases of the vehicle, negative control, extracts and Madecassol® administered animals.

Groups	Extract type	Wound healing processes								Healing phases		
		S	U	RE	FP	CD	MNC	PMN	NV	I	P	R
Vehicle		+++	+++	–	++/+++	++/+++	++	++	++/+++	++/+++	++/+++	–
Negative control		++/+++	++/+++	–	++/+++	++/+++	++/+++	++/+++	++/+++	++/+++	++/+++	–
Ranunculus pedatus	*n*-Hexane	++/+++	–/+	–/+	++	++	++	++	++	++	++	–/+
	Diethylether	++/+++	++/+++	–	++/+++	++	++	++	++/+++	++/+++	++/+++	–
	Ethyl acetate	++	–/+	–/+	++	++	++	++	++	++	++	–/+
	Aqueous	++	–	+/++	++	+/++	+/++	+/++	++	+/++	++	+/++
	Methanol	++	–	++	++	++	+/++	+/++	++	++	++	+
Ranunculus constantinapolitanus	*n*-Hexane	++/+++	++/+++	–	++	++	++	++	++/+++	++	++	–
	Diethylether	++/+++	++/+++	–	++	++	++	++	++	++/+++	++	–
	Ethyl acetate	++/+++	++	–	++/+++	++/+++	++	++	++/+++	++	++/+++	–
	Aqueous	++	–	+	++	++	+/++	+/++	++	+/++	++	+
	Methanol	++/+++	–/+	–/+	++	++	++	++	++/+++	++	++/+++	–/+
Madecassol®		++	–	+/++	++	+/++	+/++	+/++	+/++	+/++	++	+/++

HE and VG stained sections were scored as absent (–), mild (+), moderate (++) and severe (+++) for epidermal and/or dermal re-modeling. S: scab, U: ulcus, RE: re-epithelization, FP: fibroblast proliferation, CD: collagen depositions, MNC: mononuclear cells, PMN: polymorphonuclear cells, NV: neovascularization, I: inflammation phase, P: proliferation phase, and R: re-modeling phase.

3. Results and discussion

Wound healing process comprises of three phases inflammation, proliferation and remodeling (Kondo, 2007). Agents which contribute wound healing in one or more phases are needed for the activity enhancement. In the present study, we aimed to evaluate the wound healing activities of various extracts prepared from *Ranunculus pedatus* and *Ranunculus constantinapolitanus* by using *in vivo* incision and excision wound models subsequently histopathological analysis and hydroxyproline estimation. Anti-inflammatory activity of the extracts was also assessed. The experimental results are presented in Tables 2–6.

As shown in Table 2, methanolic extract of *Ranunculus pedatus* displayed significant effect in incision wound model with the value of 31.4%. Moreover, the same extract treated group animals showed remarkable wound healing effect in excision wound model (Table 3). The contraction values were recorded as 31.08% and 55.74% on days 10 and 12 respectively. In addition, the methanolic extract of *Ranunculus constantinapolitanus* demonstrated significant effect in the same wound model.

In order to score the epidermal or dermal re-modeling, re-epithelization or ulcus in epidermis; fibroblast proliferation, mononuclear and/or polymorphonuclear cells, neo-vascularization and collagen depositions in dermis were analyzed. Re-epithelization was completed in the group treated with the methanolic extract of *Ranunculus pedatus*. Phases in wound healing processes (inflammation, proliferation, and remodeling) were observed within the experimental groups with different degree (Table 4 and Fig. 1). The reference drug and methanolic extract of *Ranunculus pedatus* treated group demonstrated faster re-modeling compared to the other groups. Limited wound healing processes were seen in the groups treated with the rest of the extract ointments. Negative control and vehicle treated groups showed delayed wound healing.

Hydroxyproline estimation is an important parameter for wound healing which gives an idea of collagen concentration that provides an enhancement to the strength of healed tissue. The hydroxyproline contents of extract treated tissues are presented in Table 5. Methanolic and aqueous extracts of *Ranunculus pedatus* and methanolic extract of *Ranunculus constantinapolitanus* treated groups showed significant enhancement in hydroxyproline content.

Table 5
Effect of topical treatment of the test ointments for 7 days on hydroxyproline content.

Material	Extract type	Hydroxyproline (μg/mg) ± S.E.M.
Negative control		22.3 ± 2.03
Vehicle		25.2 ± 2.44
Ranunculus pedatus	*n*-Hexane	23.5 ± 2.41
	Diethylether	23.9 ± 3.12
	Ethyl acetate	26.2 ± 3.03
	Methanol	**46.1 ± 3.23****
	Aqueous	**32.4 ± 2.82***
Ranunculus constantinapolitanus	*n*-Hexane	18.3 ± 2.51
	Diethylether	20.2 ± 2.63
	Ethyl acetate	28.1 ± 2.85
	Methanol	**42.7 ± 2.15****
	Aqueous	25.2 ± 3.24
Madecassol®		**60.8 ± 3.63*****

S.E.M.: standard error of the mean.
* $p < 0.05$ significant from the control.
** $p < 0.01$ significant from the control.
*** $p < 0.001$ significant from the control.

Anti-inflammatory activity is essential for the wound healing process, since long period in inflammatory phase results in retardation of healing. Methanolic extracts of both *Ranunculus pedatus* and *Ranunculus constantinapolitanus* were found to be effective in anti-inflammatory activity assessment assay. According to Table 6, the activity value of *Ranunculus pedatus* (26.2%) was higher than that of *Ranunculus constantinapolitanus* (23.3%).

ethyl acetate extract of *Ranunculus pedatus*, (6) *Ranunculus pedatus* methanol group, 10 day old wound tissue treated with the methanol extract of *Ranunculus pedatus*, (7) *Ranunculus pedatus* water group, 10 day old wound tissue treated with the aqueous extract of *Ranunculus pedatus*, (8) *Ranunculus constantinapolitanus* diethylether group, 10 day old wound tissue treated with the diethylether extract of *Ranunculus constantinapolitanus*, (9) *Ranunculus constantinapolitanus n*-hexane group, 10 day old wound tissue treated with the *n*-hexane extract of *Ranunculus constantinapolitanus*, (10) *Ranunculus constantinapolitanus* ethyl acetate group, 10 day old wound tissue treated with the ethyl acetate extract of *Ranunculus constantinapolitanus*, (11) *Ranunculus constantinapolitanus* methanol group, 10 day old wound tissue treated with the methanol extract of *Ranunculus constantinapolitanus*, (12) *Ranunculus constantinapolitanus* water group, 10 day old wound tissue treated with the aqueous extract of *Ranunculus constantinapolitanus*, and (13) reference group, 10 day old wound tissue treated with Madecassol®. Arrows pointing events during wound healing; s: scab, u: ulcus, re: re-epithelization, f: fibroblast, c: collagen, mnc: mononuclear cells, pmn: polymorphonuclear cells, and nv: neovascularization.

Table 6
Inhibitory effect of the extracts of *Ranunculus pedatus* and *Ranunculus constantinapolitanus* on acetic acid-induced increased in capillary permeability.

Material	Extract type	Dose (mg/kg)	Evans blue concentration (μg/ml) ± S.E.M.	Inhibition (%)
Control			9.40 ± 1.20	
Ranunculus pedatus	*n*-Hexane	100	9.25 ± 1.16	1.6
	Diethylether	100	10.21 ± 1.32	–
	Ethyl acetate	100	7.66 ± 1.13	18.5
	Methanol	100	6.94 ± 0.71	**26.2**[*]
	Aqueous	100	7.86 ± 1.01	16.4
Ranunculus constantinapolitanus	*n*-Hexane	100	9.44 ± 1.15	–
	Diethylether	100	9.19 ± 1.23	2.2
	Ethyl acetate	100	8.11 ± 1.09	13.7
	Methanol	100	7.21 ± 0.69	**23.3**[*]
	Aqueous	100	8.45 ± 0.98	10.1
Indomethacin		10	3.77 ± 0.30	**59.9**[***]

S.E.M.: standard error of the mean.
[*] $p < 0.05$ significant from the control.
[***] $p < 0.001$ significant from the control.

Preliminary phytochemical screening of different chemical compounds (steroids, triterpenoids, alkaloids, tannin, anthraquinone, sugars, reducing sugar, flavonoids, coumarins, starchs, saponins) was analyzed in the extracts. Thus out of ($10 \times 10 = 100$) tests for the presence or absence of the above compounds, only 12 gave positive results (Table 1). Alkaloids are present only in diethylether extracts, saponins are present in water extracts. Flavonoids are present in both ethyl acetate and methanol extracts. Among the five different extracts of plants only the methanol extracts show the presence of steroids, triterpenes and flavonoids.

In reviewing the literature, we highlighted the role of phenols, flavonoids and terpenoids in the treatment of inflammation and wound healing (Talhouk et al., 2007). Previous reports revealed that terpenoids have multiple pharmacological effects (Edris, 2007). Some studies have shown that terpenoids have cytotoxic, lipophilic, bactericidal, fungicidal, insecticidal, anticarcinogenic, pesticidal, antioxidant, anti-inflammatory and analgesic properties (Takayama et al., 2011). Furthermore, terpenoids are known to promote the wound healing process, because of their astringent and antimicrobial effects, which seem to be responsible for the wound contraction, therefore an increased rate of epithelialization (Scortichini and Pia Rossi, 1991; Sasidharan et al., 2010). Flavonoids are also used for their several therapeutic effects such as antioxidant, antiinflammatory, antifungal and wound healing (Okuda, 2005; Nayak et al., 2009). Inhibition of lipid peroxidation effect by flavonoids results in the increase of the viability of collagen fibrils and prevention of the cell damage (Getie et al., 2002; Shetty et al., 2008). Flavonoids are also known to promote the rapid wound healing due to their antimicrobial and astringent properties (Tsuchiya et al., 1996). Accordingly, the methanol extracts of the plants which presumably rich in these compounds was found to be active in wound healing and anti-inflammatory activity evaluation assays. In conclusion, wound-healing potential of *Ranunculus pedatus* may be attributed to the flavonoids and terpenoids present in the aerial parts, which may be either due to their individual or additive effect that accelerates the healing process. Further phytochemical studies are in progress, where the methanolic extract will be subjected to further fractionation for the identification of the compound(s) responsible for the activity. This study provides scientific evidence for the ethnomedicinal features of *Ranunculus* species in Turkey.

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